

## LABORATORY ANIMAL PROJECT REVIEW

**Please note:**

1. All information in this LAPR is considered privileged and confidential by the IACUC and regulatory authorities.
2. Approved LAPRs are subject to release to the public under the Freedom of Information Act (FOIA). Do not include proprietary or classified information in the LAPR.
3. An approved LAPR is valid for three years.

### LAPR Information

**LAPR Title:** Priming Effects of Air Pollution Exposure on Cardiopulmonary Responses After a Single Oral Fat Load in Rats  
**LAPR Number:** 19-01-003  
**Principal Investigator:** Exemption 6  
**Author of this Document:** Exemption 6/RTP/USEPA/US  
**Date Originated:** 11/05/2015  
**LAPR Expiration Date:** 01/31/2019  
**Agenda Date:** 12/02/2015  
**Date Approved:** 01/05/2016  
**Date Closed:**

### APPROVALS

APPROVER	NAME	APPROVAL DATE	COMMENTS	
	Exemption 6/RTP/USEPA/US by Exemption 6/RTP/USEPA/US	01/05/2016	DMR	
	Exemption 6 Exemption 6 Exemption 6/RTP/USEPA/US by Exemption 6/RTP/USEPA/US	01/05/2016	DMR approved	


### Administrative Information

**1. Project Title (no abbreviations, include species):**

Priming Effects of Air Pollution Exposure on Cardiopulmonary Responses After a Single Oral Fat Load in Rats

**Is this a continuing study with a previously approved LAPR?**

No

**2. Programmatic Information**

**a. What Program does this LAPR support? Please provide the Research Program, Project, Task Number and Title.**

Air Climate and Energy (ACE) Research Action Plan (PEP Tasks 1.1, 1.2, 1.3, and 2.2).

**b. What is the Quality Assurance Project Plan (QAPP) covering this project?**

IRP-NHEERL-RTP/EPHD/CIB<sup>Exemption 6</sup>/15-01-001 (see attachment below)

**3. EPA Principal Investigator/Responsible Employee:**

<b>Principal Investigator</b> <b>Exemption 6</b>	<b>Phone Number</b> <b>Exemption 6</b>	<b>Division</b> EPHD	<b>Mail Drop</b> MD
	<b>Lotus Notes Address</b> <b>Exemption 6</b> <b>Exemption 6</b> <b>Exemption 6</b>	<b>Branch</b> CIB	
	RTP/USEPA/US		

**4. Alternate Contact:**

<b>Alternate Contact</b> <b>Exemption 6</b>	<b>Phone Number</b> <b>Exemption 6</b>	<b>Division</b> EPHD	<b>Mail Drop</b> MD
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	RTP/USEPA/US		

### SECTION A - Description of Project

**1. Explain the study objective(s) in non-technical language such that it is understandable by non-scientific persons. Explain how the benefits from the knowledge gained from this research outweigh the costs to the animals used in this research. If this is a continuing study from a previous LAPR, briefly justify the continuation. Please spell out all acronyms and abbreviations with their initial use.**

Multiple studies have shown that air pollution exposure primes body systems to heightened responses to everyday activities that stress the cardiovascular system (e.g. exercise). For example, in previous studies, we've determined that air pollution exposure causes exaggerated cardiovascular responses to treadmill exercise and to a drug that causes cardiac arrhythmia in rats. Thus, low exposures may elicit subtle effects that may not manifest in the absence of a stressor. Moreover, in both healthy people and individuals with pre-existing cardiovascular disease, a single high fat meal is also a transient stressor of the cardiovascular system causing immediate potentially deleterious impacts including altered function of blood vessels, increases in bad cholesterol and triglycerides, and altered insulin sensitivity and pulmonary function. Pre-exposure to air pollution may similarly exaggerate cardiopulmonary responses to a single high fat meal. The goal of these studies is to investigate the impacts of exposure to air pollution on the cardiovascular effects after a single high fat meal (also called post-prandial effects). Multiple studies have already determined that post-prandial assessments of circulating lipids and fats in people are better predictors of cardiovascular risk than measures in fasting individuals. This task will provide research that directly addresses the following explicitly stated needs of the United States Environmental Protection Agency's (US EPA) Office of Air and Radiation: 1) Increased understanding of true multipollutant effects/impacts and 2) Increased understanding of the potential benefit and/or harm of modifiable factors and identification of intervention strategies to mitigate the health effects of air pollution. This research is part of the US EPA's Air Climate and Energy (ACE) Research Action Plan (PEP (Protection of the Environment and Public Health) Tasks 1.1, 1.2, 1.3, and 2.2).

We will model a single high fat meal in rats by administering, via a single oral gavage, a bolus high fat emulsion (a high fat chow suspended in water). All rats will receive oral gavage after exposure to air pollution. Pilot studies will be run to assess the cardiovascular effects of high fat gavage in naïve un-exposed rats. Subsequent studies will assess the priming effects of a single air pollution exposure and repeated air pollution exposure on cardiovascular responses. The role of several putative mechanisms in these responses will be examined. One mechanism hypothesizes that the exaggerated cardiovascular effects of air pollution are due to enhancement of systemic lipids and will be tested using a statin, a well-documented lipid-lowering class of drugs. Epidemiological studies have documented that individuals who take statins have reduced air pollution health effects. The second mechanism involves the use of genetic knockout rats that are missing a functional nerve growth factor (NGF) receptor (i.e. p75 <sup>-/-</sup> rats). Studies have shown that NGF influences cardiac pathophysiology.

Male Wistar-Kyoto or Sprague Dawley rats will be exposed to air pollution as often as twice per week for up to 3 months. Rats will be co-exposed via whole body exposure to concentrated ambient particulate matter (CAPs) and Nitrogen Dioxide. Rats will receive an oral gavage one day after one exposure and one day after the final exposure in the long term exposure study. Echocardiography (i.e. noninvasive ultrasound assessment of cardiac structure and mechanical function), implantable blood pressure and electrocardiogram radiotelemetry (survival surgeries will be performed by Charles River Laboratories - see attachment), and whole body plethysmography (an approach used to assess breathing/ventilation) will be used to assess cardiopulmonary function 2 to 4 hours after gavage. Systemic lipid and inflammatory responses in blood, lung, heart, and liver tissue will also be determined. Sensitivity to aconitine-induced arrhythmia will also be assessed after gavage (aconitine is a cardiotoxic substance widely used to produce cardiac arrhythmia). This assay is a terminal surgical procedure that measures how sensitive the subject is to a stressor and is thus a measure of cardiac vulnerability.

## **2. Scientific rationale for proposed animal use.**

### **a. Why is the use of animals necessary?**

The adverse effects ascribed to air pollution exposure result from the interplay and summation of responses across multiple organ systems, with one deficient organ system impacting others. Thus, it is requisite that whole animals be used for these studies.

### **b. Justify the species requested:**

The rat is the species of choice for our studies because:

- 1) Several rat strains mirror human cardiopulmonary responses to air pollution.
- 2) There is a very large toxicological database using the rat as the subject species and this will provide the capability to compare toxicity data within a given species.
- 3) Nearly all of the earlier air pollution studies conducted in our laboratory have used the rat as the animal model and continuing this will permit comparisons with the results of our previous studies.

## **3. How was it determined that this study is not unnecessary duplication?**

Searches of the entire air pollution health research database via Pub-Med using one or more of the search terms air pollution, single high fat meal, post-prandial, cardiovascular, rats, echocardiography, oral gavage,

statins, nerve growth factor, telemetry, aconitine challenge (conducted on November 17, 2015), yielded no studies similar to those proposed below; thus, these studies will not duplicate any previously conducted research.

## **SECTION B - In Vivo Procedures**

***1. Briefly describe the experimental design. Include descriptions of the age, weight and sex of the animals. Supplementary information may be attached at the end of the LAPR, but please include critical information within the body of the LAPR.***

All studies will use male rats 10-12 weeks old and 250-350 grams. The first three studies will use Wistar Kyoto rats. The final study will use two rat strains: 1) p75 knockout rats that were derived from Sprague Dawley rats and 2) Sprague Dawley rats as background controls.

The initial study will assess the cardiovascular effects of high fat and low fat gavage in naïve un-exposed rats using echocardiography, aconitine challenge (a drug that causes cardiac arrhythmia), and assessments of systemic lipids. Note: These rats will not be implanted with telemeters.

The subsequent study will compare the priming effects of a single exposure to CAPs and NO<sub>2</sub> on cardiovascular responses in rats that are administered an oral gavage of a high fat emulsion versus rats that receive an oral gavage of a vehicle control (i.e. water). Physiological (echocardiography, aconitine challenge) and systemic indicators will be assessed up to 4 hours after gavage challenge. Note: These rats will not be implanted with telemeters.

The third study will assess the priming effects of long term air pollution exposure on the cardiopulmonary effects of a single high fat meal in rats treated with/without a statin, a well-documented lipid-lowering class of drugs. The statin to be used is pitavastatin, a drug used clinically to lower serum levels of total cholesterol, bad cholesterol (LDL (low density lipoprotein)-cholesterol, and triglycerides, and raise levels of good cholesterol (HDL (high density lipoprotein)-cholesterol). Rodents will be exposed to CAPs and NO<sub>2</sub> twice a week for up to 3 months. One day following the first exposure (to assess short term effects) and one day after the last exposure, rats will be receive an oral gavage of a high fat emulsion. Physiological and systemic indicators will be assessed up to 4 hours after gavage. Echocardiography, radiotelemetry, aconitine challenge, and whole body plethysmography will be used to assess cardiopulmonary function. Systemic lipid and inflammatory, responses will also be determined.

The fourth study will mirror the third study in design with the exception that NGF receptor knockout rats (p75 -/-) and background Sprague Dawley controls will be used. Studies have shown that NGF influences cardiac pathophysiology.

***2. Justify the number of animals. Include explanation (e.g., biological, statistical, regulatory rationale) for the number of animals needed for each treatment group, and the overall number requested for the duration of the LAPR.***

We have determined from previous studies that a group size of 8 allows for sufficient power in the statistical analysis of various physiological endpoints. All studies will involve oral gavage administration of a water suspendable rodent diet from Research Diets (see attachment). The first study will compare the effects of two different emulsions that are equal in total calories, but differ in % fat: 1) a 1 kcal/ml emulsion that derives 10% of the calories from fat (i.e. 10 kcal%) and 2) a 1 kcal/ml emulsion that derives 60% of the calories from fat (i.e. 60 kcal%). All subsequent studies will use only the 60 kcal% emulsion for oral gavage. The following is a description of exposure groups and animal numbers for each of the studies:

Study 1: Pilot study to determine effects of oral gavage of high fat emulsion on cardiovascular responses in naive unexposed rats (Note: No air pollution exposure and no telemetry):

- Water gavage: = 8 x 2 cohorts (echo/lipids and aconitine) = 16
- 10 kcal% Fat gavage: N = 8 x 2 cohorts (echo/lipids and aconitine) = 16
- 60 kcal% Fat gavage: N = 8 x 2 cohorts (echo/lipids and aconitine) = 16

Note 1: Un-telemetered 10-12 week old male WKY rats will be used.

Note 2: Rats will receive an oral gavage of a 10 ml/kg of 1 kcal/ml emulsion or water.

Note 3: Echo rats will undergo ultrasound scanning for echocardiographic analysis 2 to 4 hours after gavage and then will be euthanized immediately after ultrasound with subsequent assessment of systemic lipids.

Note 4: A separate cohort of animals are included for aconitine challenge because aconitine is quite toxic and thus we prefer not to collect tissue from rats treated with it. Aconitine rats will undergo terminal aconitine challenge 2 to 4 hours after gavage.

Total Rats = 48 (24 Cat. C rats (echo) and 24 Cat. D rats (aconitine))

Study 2: Interactive Effects of Single Exposure to Air Pollution and High Fat Gavage on Systemic Responses, Echocardiography and Aconitine Challenge (Note: No telemetry):

- Water gavage - Filtered Air: N = 8 x 2 cohorts (echo/lipids and aconitine) = 16
- Water gavage - Air Pollution: N = 8 x 2 cohorts (echo/lipids and aconitine) = 16
- 60 kcal% Fat gavage - Filtered Air: N = 8 x 2 cohorts (echo/lipids and aconitine) = 16
- 60 kcal% Fat gavage - Air Pollution: N = 8 x 2 cohorts (echo/lipids and aconitine) = 16

Note 1: Un-telemetered 10-12 week old male WKY rats will be used.

Note 2: Responses will be assessed after a single 4 hr whole body exposure to CAPs and NO<sub>2</sub>.

Note 3: All rats in the study will receive an oral gavage of a high fat emulsion or water alone as in table 1 one day after a single whole body exposure to air pollution.

Note 4: Echo rats will undergo ultrasound scanning for echocardiographic analysis 2 to 4 hours after gavage followed by necropsy and systemic lipid assessments.

Note 5: Aconitine rats will undergo terminal aconitine challenge 2 to 4 hours after gavage.

Total Rats = 64 (32 Cat. C rats (echo) and 32 Cat. D rats (aconitine))

Study 3: Long-term Air Pollution Exposure and Statin Study with Gavage Challenge and Implantable telemetry (all rats will receive high fat challenge)

- Statin - Filtered Air: N = 8 x 2 cohorts (telemetry and post-single exposure) = 16
- Statin - Air Pollution: N = 8 x 2 cohorts (telemetry and post-single exposure) = 16
- Saline - Filtered Air: N = 8 x 2 cohorts (telemetry and post-single exposure) = 16
- Saline - Air Pollution: N = 8 x 2 cohorts (telemetry and post-single exposure) = 16

Note 1: 10-12 week old male WKY rats will be used; half of the rats (n=32) will be telemetered.

Note 2: For statin treatment, rats will be orally gavaged with up to 5 mg/kg of pitavastatin or vehicle control (0.5% carboxymethylcellulose) one day before each inhalation exposure to air pollution.

Note 3: Rats will be exposed whole body twice per week (4h/day) for up to 6 months to CAPs (maximum concentration = 500 micrograms/m<sup>3</sup>) and NO<sub>2</sub> (maximum concentration = 0.5 ppm).

Note 4: All telemetered rats will receive an oral gavage of a high fat emulsion as in Study 2 one day after a single whole body exposure to air pollution or filtered air and again after the final exposure.

Note 5: All telemetered rats will undergo echocardiographic analysis 2 to 4 hours after each gavage.

Note 6: Telemetered rats will be euthanized following the 2nd gavage, immediately after final echo.

Note 7: A separate cohort of non-telemetered animals are included to assess the impacts of a single exposure on cardiopulmonary responsiveness. Non-telemetered rats will be euthanized following the first gavage one day after a single exposure.

Total Rats = 64 (32 Cat. C rats (post-single exposure) and 32 Cat. D rats (telemetry))

Study 4: Role of NGF receptor p75 in Air pollution-induced Post-prandial Physiological and Systemic Responses (all rats receive high fat challenge)

- SD Rats - Filtered Air: N = 8 x 2 cohorts (telemetry and post-single exposure) = 16
- SD Rats - Air Pollution: N = 8 x 2 cohorts (telemetry and post-single exposure) = 16
- p75 (-/-) Rats - Filtered Air: N = 8 x 2 cohorts (telemetry and post-single exposure) = 16
- p75 (-/-) Rats - Air Pollution: N = 8 x 2 cohorts (telemetry and post-single exposure) = 16

Note 1: Rats will be exposed whole body twice per week (4h/day) for up to 6 months to CAPs (maximum concentration = 500 micrograms/m<sup>3</sup>) and NO<sub>2</sub> (maximum concentration = 0.5 ppm).

Note 2: All telemetered rats will receive an oral gavage of a high fat emulsion as in Study 2 one day after a single whole body exposure to air pollution or filtered air and again after the final exposure

Note 3: All telemetered rats will undergo echocardiographic analysis 2 to 4 hours after each gavage.

Note 4: Telemetered rats will be euthanized following 2nd gavage, immediately after final echo.

Note 5: Non-telemetered rats will be euthanized following the first gavage one day after a single exposure.

Total Rats = 64 (32 Cat. C rats (post-single exposure) and 32 Cat. D rats (telemetry))

Total rats for all studies = 240 (120 Cat. C and 120 Cat. D)

Please note that all rats designated for echocardiography assessments are designated under Pain Category C, while those planned for telemetry or aconitine challenge, both surgical procedures, have been designated Category D.

3. State how many animals over the study period are expected to be used under the following three categories of pain/distress (USDA nomenclature as defined in the instructions ): Please enter numbers only.

Categories	Adults	Offspring
C) Minimal, transient, or no pain/distress:	120	
D) Potential pain/distress relieved by appropriate measures:	120	
E) Unrelieved pain/distress:		

4. Does this LAPR include any of the following:

- ☐ Restraint (>15 Minutes) ☒ Survival surgery  
☒ Food and/or water restriction (>6 Hours) ☒ Non-survival surgery

a. Please provide a scientific justification. Describe how animals will be monitored, how health status will be tracked, and what records will be maintained.

Implantable telemetry (a Survival Surgery) is the only method available that enables the acquisition of reliable unconfounded electrocardiogram and blood pressure data in conscious unrestrained animals before, during and after exposure (see section B9 for search terms). Rats will be delivered to the EPA from Charles River approximately 10 days after surgery and housed in Room **Exemption 6**. Staples will be removed a couple of days after arrival while under isoflurane anesthesia. Animals will be monitored visually (obvious distress, gait, breathing, appetite, etc.) at least twice daily. Animals will be weighed on a regular basis and tracked for sudden weight loss (>10%). Animals will be continuously monitored telemetrically for signs of decrements in physiological function. The personnel responsible for this care include: **Exemption 6**  
**Exemption 6** Written records of surgical and post-surgical care will be maintained by **Exemption 6** in room A463-A.

Intravenous infusion (a terminal surgery) is the only method available that will allow metered dosing of aconitine with direct access to the heart. The rats will be monitored during the entire treatment regimen and then euthanized via injection with pentobarbital as described below.

5. Category C procedures. Describe each procedure separately, include details on the following:

a. Treatments (e.g., dosages, duration of exposure, route, volume, frequency):

Oral Gavage Administration of High Fat Emulsion: Male rats (10-12 weeks old and weighing 250-350 grams) fasted overnight will receive up to 10 ml/kg (3 ml for a 300 gram rat) of a 1 kcal high fat emulsion (water suspendable rodent diet from Research Diets - see attachment) in the morning. Rats will receive an oral gavage of one of two emulsions that are equal in total calories, but differ in % fat: 1) a 1 kcal/ml emulsion that derives 10% of the calories from fat (i.e. 10 kcal%) and 2) a 1 kcal/ml emulsion that derives 60% of the calories from fat (i.e. 60 kcal%). Rats typically consume approximately 12 kcal per meal (~ 3 grams of chow). (Note: A 2 kcal/ml emulsion may be used if it's deemed that the 1 kcal/ml emulsion doesn't elicit appreciable effects on systemic lipids and cardiac function.) Manually restrained (unanesthetized) rats will be gavaged using a 14G gavage feeding needle that is 3 to 4 inches in length and has a 4 mm ball diameter.

Oral Gavage Administration of Statin: The third study will assess the priming effects of long term air pollution exposure on the cardiopulmonary effects of a single high fat meal in rats treated with/without a statin, a well-documented lipid-lowering class of drugs. The statin to be used is pitavastatin, a drug used clinically to lower serum levels of total cholesterol, bad cholesterol (LDL (low density lipoprotein)-cholesterol, and triglycerides, and raise levels of good cholesterol (HDL (high density lipoprotein)-cholesterol). Rats will receive an oral gavage of 5 mg/kg of pitavastatin or vehicle control (dimethylsulfoxide or carboxymethylcellulose) one day before each inhalation exposure to air pollution using a 14G gavage feeding needle that is 3 to 4 inches in length and has a 4 mm ball diameter.

Note: Animal Care Staff will be requested to assist with the oral gavage procedure.

Whole-body Co-exposure to CAPs and Nitrogen Dioxide (NO<sub>2</sub>): Animals will be exposed via whole-body inhalation to CAPs and NO<sub>2</sub> for 4 hours twice per week for up to 3 months in exposure chambers of stainless steel and glass construction and animals will be individually housed in cage sets. Approximately 10,000 Lpm outside ambient air containing PM will be drawn into concentrating equipment in Exemption 6  
Exemption 6. Prior to entering the lab, air passes thru a size selective inlet assembly where particles larger than 2.5 µm are removed. PM will be concentrated using slit virtual impactors. Chamber concentrations will typically average around 27-times ambient levels in the chamber. The maximum exposure concentration for PM will be 500 micrograms/m<sup>3</sup>. These concentrations are based either on PM mass (µg/m<sup>3</sup>) or on particle count (#/cc). NO<sub>2</sub> gas will be metered in to the chamber from a stock gas cylinder source. The maximum exposure concentration for NO<sub>2</sub> will be 0.5 ppm. The control system will include appropriate safety valves and gas flow metering devices to allow either manual or automatic injection of NO<sub>2</sub> into chamber supply air upstream of the exposure chamber. The chambers will be opened and animals removed at least 5 minutes after the exposure has terminated. The control chamber animals will be exposed to HEPA filtered air (FA). Chamber temperature, relative humidity, and pressure, will be continuously monitored and recorded. Rats will be visually monitored at least once every hour during exposure and up to two hours after exposure.

Note: Given that CAPs and NO<sub>2</sub> co-exposures have not been carried out before in our inhalation facility, in the event of the inability to perform co-exposures, rats may be exposed to CAPs alone or NO<sub>2</sub> alone (same exposure concentrations and duration) as an alternative.

***b. Survival Blood Collections (method, volume, frequency):***

***c. Testing methods (including non-stressful dietary restrictions/modifications, mild non-damaging electric shock):***

Ultrasound echocardiography: Cardiac function will be determined via HF Echo using a Visual Sonics Vevo 2100 ultrasound system in room Exemption 6  
Exemption 6. Rats will be placed in a sealed chamber and anesthetized with 2-5% Isoflurane delivered in 100% O<sub>2</sub> at 0.8-1 L/min. We will then move the rats to a heated procedure table where anesthesia will be maintained with 1-4% Isoflurane (100% O<sub>2</sub> @ 0.8-1 L/min) via a nose cone. The eyes will be coated with eye lubricant to prevent drying of the eyes during the procedure. Each paw will be gently taped to ECG electrodes coated with electrode gel (to monitor heart rate and respiratory rate) and body temperature will be monitored with a rectal probe. Nair gel will be used to remove fur from the imaging location (chest and upper abdomen). The application area will be washed to remove any residual Nair from the skin. Pre-warmed ultrasound gel will be applied to the chest and the HF Echo transducer will be used to noninvasively record 3 video loops of blood flow into the left ventricle, blood flow through the pulmonary artery, and the contractile motion of the heart. Each of these measurements will be made one day after exposure after a single exposure (both single exposure and long term studies) and one day after the final exposure in the long term exposure studies. After the measurements are made, rats will be gently and carefully cleaned of all transducer gel. Rats will then be removed from anesthesia and allowed to recover in fresh clean cages. Rats will be monitored during the recovery period until normal grooming habits resume before returning to animal holding room Exemption 6  
Exemption 6.

Note: Precautions will be taken to ensure that freshly clean cages do not come into contact with any lab surface during the ultrasound procedure. Rats will then be euthanized for necropsy after the final echo.

Ventilatory monitoring (unanesthetized): Selected rats will undergo ventilation monitoring using the unanesthetized, unrestrained Buxco Exposure System. Rats will be placed in whole-body plethysmographs (WBP) and monitored for breathing frequency, tidal volume, minute ventilation, Penh, etc. before and after air

pollution exposure and/or oral gavage. There will be 1-2 days allowed for acclimation in the WBP prior to exposure, while post-exposure pulmonary monitoring sessions will last 10 min/day. Chamber temperature, humidity, and air flow will be continuously monitored throughout the WBP sessions. Food and water will be restricted during the WBP sessions. Subsets of animals will be monitored telemetrically for signs of distress.

**d. Animal restraint and confinement beyond routine housing and handling. Include a description of the type of restraint device, acclimation to device, duration of restraint:**

**e. Breeding for experimental purposes (e.g. length of pairing, number of generations):**

**f. Describe how animals will be identified and monitored. Include description of identification procedures. (For example, if transponders are used, how are the animals prepared?) Include frequency of observations and by whom:**

Implantation of identity chips: Identity chips will be inserted subcutaneously in the dorsal neck area using isoflurane anesthesia (~ 1 min) by the animal care staff using a sterile 16 gauge needle before 3-5 days before other surgical procedures. These chips have unique serial numbers that will be linked with a uniquely numbered rat to ensure proper identification of the rats.

Whole body air pollution exposures: Rats will be visually monitored at least once every hour during exposure and up to two hours after exposure by **Exemption 6Exemption 6Exemption 6Exemption 6**.

Ultrasound echocardiography: The Vevo 2100 Ultrasound system comes equipped with an electrocardiogram (ECG) platform on which the anesthetized animal will be placed and monitored. This will enable measurement of ECG, heart rate, and respiratory rate by the individual performing the assessments (i.e. **Exemption 6Exemption 6Exemption 6**). The system also includes a rectal probe to measure internal body temperature. All values will be prominently displayed on the screen enabling monitoring from start to finish. After removing the rat from the anesthesia induction chamber, eye ointment and Nair are applied and each limb paw will be placed on an electrode contact to monitor ECG, heart and respiratory rates. The rectal probe will then be inserted to monitor body temperature. All vital signs will be monitored until the animal is returned to its home cage in the case of pre-exposure assessments or in the instance of euthanasia, administration of sodium pentobarbital.

Ventilatory monitoring: Rats will be visually monitored at least once every hour during procedure and up to two hours after the procedure by **Exemption 6Exemption 6Exemption 6Exemption 6**.

Oral gavage: Rats will be monitored post gavage for potential complications including evidence of damage to the oral cavity, esophagus or trachea, insertion of needle and solution into the lungs or aspiration of solution into the lungs from regurgitation, or injury to the animal from restraint. Animals will be re-checked for injury one hour after the procedure by **Exemption 6Exemption 6Exemption 6Exemption 6**.

**6. Non-surgical Category D or E procedures. Describe each procedure separately, include details on the following (Also fill in Section B.9).**

**a. Treatments (e.g. dosages, duration of exposure, route, volume, frequency):**

**b. Blood Collection (Provide a description of the procedure including method, volume, and frequency if appropriate. Indicate if the procedure is survival or terminal. Include preparatory methods, descriptions of incisions, etc.):**

**c. Testing methods:**

**d. Restrictions placed on the animals' basic needs (e.g., food and/or water restriction, light cycles, temperature). Provide details regarding the length of restriction. Describe the method(s) for assessing the health and well-being of the animals during restriction. (Amount of food or fluid earned during testing and amount freely given must be recorded and assessed to assure proper nutrition.):**

**e. Describe how animals will be monitored (e.g., frequency of observations, by whom):**

**f. Analgesia (Category D Procedures) - list drugs, dosages, route of administration and frequency:**

**g. If treatment-related deaths are expected, this must be thoroughly justified. Death as an endpoint is highly discouraged:**

**7. Surgical Category D and E procedures. Indicate if the surgery is survival or terminal. Describe each surgical procedure separately, include details on the following (Also fill in Section B.9)**

**a. Complete description of surgical procedure including presurgical preparation, aseptic technique, surgical closure, etc:**

Surgical Implantation of Blood pressure telemeters:

All blood pressure telemetry surgeries in this protocol will be done by Charles River Laboratories (CRL; Wilmington, MA). CRL has their own internal institutional IACUC approval (please see attachment). Animals will be anesthetized and implanted with sterile radiotelemetry transmitters. The procedures to be followed are essentially similar to those described in previous publications (Watkinson et al., J. Appl. Physiol. 78:1108-1120, 1995; Watkinson et al., Toxicological Sciences 41:209-216, 1998).

Procedure for reattachment of telemetry leads:

In the event that sutured leads come undone, they will be reattached with suture in the surgical suite. The Attending Veterinarian will be notified for additional guidance. Aseptic technique will be observed for this procedure, including the use of sterilized instruments (autoclaving), surgical gloves, surgical masks and suture material. The following aseptic procedure will be followed: Rats will be anesthetized with isoflurane (3% isoflurane, 97% oxygen). Body temperature will be maintained throughout the entire procedure with a circulating heating pad. The ventral surface by the right shoulder or area to the left of the xiphoid space (sites of subcutaneous lead attachment) will be shaved using electrical clippers (Oster, Detachable Blade Animal Clipper; Model #5-01, Series SM03), scrubbed with disinfectant solution (Betadine Solution), and cleansed with alcohol (PDI Alcohol Prep Pads). After an appropriate anesthetic plane is achieved (unresponsive to foot pinch), the leads will be reattached with suture and skin closed using surgical staples. Postoperative antibiotic wound dressings (10% povidone-iodine ointment) will be applied. Animals will then be returned to their cages.

Aconitine challenge arrhythmia sensitivity test (terminal procedure):

On the day of the challenge, 24 hours following the last exposure as described in the regimen above, intravenous aconitine challenge will be performed. Rats will be anesthetized with urethane (1.5 g/kg, i.p.) and surgically catheterized into the left jugular vein with saline-filled PE50 tubing for administration of drugs. The experiments will be performed immediately following implantation of the i.v. catheter. Body temperature will be supported during and after surgery. 10 micrograms/ml aconitine (in saline) will be infused into the jugular vein at a speed of 0.2 ml/min (Li et al. 2007) using a ISMATECH IPC infusion pump while electrocardiogram (ECG) is continuously monitored and timed (ECG will be monitored in these animals using an external telemeter that is attached to the skin). Susceptibility will be measured as the threshold dose of aconitine required to produce ventricular premature beats, ventricular tachycardia, and ventricular fibrillation will be calculated using the following formula:

Threshold dose (ug/kg) for arrhythmia =  $10\text{ug/ml} \times 0.2\text{ml/min} \times \text{time required for inducing arrhythmia (min)}/\text{body weight (kg)}$  =  $1\text{ug/min} \times \text{time (min)}/\text{body weight (kg)}$

The dose of aconitine administered will be calculated based on the concentration of aconitine solution used and the calibrated speed of infusion. All animals will be euthanized by a lethal dose of Na pentobarbital (200 mg/kg) administered i.v. at the end of the procedure. The procedure may take as long as 30 minutes depending on how sensitive the animals are as a result of exposure.

**b. Anesthetic regimen (Drugs, dosages, volume, route of administration and delivery schedule). The use of paralytic or neuromuscular blocking agents w/o anesthesia is prohibited:**

Ketamine (80 mg/ml)/xylazine (12 mg/ml) surgical anesthetic for telemetry implantation; 1-2 ml/kg ip as

needed for complete anesthesia. 1-2 ml/kg ketamine alone to maintain anesthetic plane.

Isoflurane (3 % per unit volume air): inhaled surgical anesthetic for re-attachment of leads in the event they come undone.

Urethane (1.5 mg/kg, ip) for aconitine test. Only urethane that is within one year of procurement will be used. Working solution will be prepared on a stir plate in the hood.

**c. Postoperative care (thermal support, special feeding, responsible personnel, removal of sutures/staples, frequency and duration of monitoring including weekend and holiday care):**  
Surgical Implantation of Radiotelemetry Transmitters

Analgesic will be administered before commencement of surgery (buprenorphine, 0.05 mg/kg) and post-surgically (buprenorphine, 0.05 mg/kg × 2/day × 2 days) at Charles River Laboratories. Postoperative antibiotic wound dressings will be applied following the surgery also at Charles River. After rats are received at EPA, staples will be removed approximately 10 days post-surgery. Once at the EPA, animals will be housed in Room **Exemption 6 Exemption 6 Exemption 6**. Animals will be monitored visually (obvious distress, gait, breathing, appetite, etc.) at least twice daily. Animals will be weighed on a regular basis and tracked for sudden weight loss (>10%). Animals will be continuously monitored telemetrically for signs of decrements in physiological function. The personnel responsible for this care include **Exemption 6 Exemption 6 Exemption 6**

**Exemption 6 Exemption 6 Exemption 6** Records of surgical and post-surgical care will be maintained by **Exemption 6 Exemption 6 Exemption 6** in room A463-A.

**d. Post operative analgesics (drugs, dosage, and volume and route of administration, frequency):**  
Buprenorphine: post-operative analgesic - 0.05mg/kg administered once every 24 hours beginning immediately before surgery for a total of 2 doses and administered once immediately before re-attaching leads.

**e. Will any animal be subject to more than one surgical procedure over the course of its lifetime, either here at NHEERL or elsewhere?**

☐ Yes ☒ No

**f. Identify any surgical procedures performed at other institutions or by vendors:**  
Charles River Laboratories will perform all telemetry surgeries.

**8. Humane interventions (for treatments/procedures in all categories).**

**a. What resultant effects, if any, do the investigators expect to see following procedures or treatment? Please include transitory as well as permanent effects. Examples might include lethargy, ataxia, salivation or tremors. Indicate the expected duration of these effects.**

No overt toxic effects are expected. All animals will be monitored visually (obvious distress, gait, breathing, appetite, etc.) at least twice daily in case effects arise. Animals will be weighed every 3 days and tracked for sudden weight loss (>10%). Representative animals from each treatment group will be monitored telemetrically for adverse effects on physiological function.

**b. State the criteria for determining temporary or permanent removal of animals from the study. Describe actions to be taken in the event of deleterious effects from procedures or chemical exposures. Describe actions to be taken in the event of clinical health problems not caused by procedures or exposures.**

Criteria for temporary or permanent removal include the presence of one or more of the following: weight loss (> 10% within one week's time from arrival), labored breathing, abnormal gait, loss of appetite, lesions in and around areas of surgical incision. If signs of distress or other deleterious effects are observed, all animals from the treatment group will be isolated in a clean control atmosphere and observed for recovery trends. They may be reused for the study if recovery is demonstrated; otherwise, they will be euthanized. The attending veterinarian will be consulted to determine the appropriate course of action.

**9. Alternatives to pain and distress (Category D and E Procedures only). Provide narrative regarding the sources consulted to ascertain whether acceptable alternatives exist for potentially painful/distressful procedures. Include databases searched or other sources consulted, the date of the search and years covered by the search, and key words and/or search strategy used. Assistance with searches is available through the EPA Library Staff.**

Implantable telemetry is the only method available that enables the acquisition of reliable unconfounded

electrocardiogram and blood pressure data in conscious unrestrained animals based on a PubMed search containing the following key words: electrocardiogram, conscious, freely moving, blood pressure, rats, and heart rate. This search was conducted on December 22, 2015 and covers the years 1993 through the present (only 6 publications found, all of which confirm these statements).

Intravenous infusion is the only method available that will allow metered dosing of aconitine with direct access to the heart based on a PubMed search containing the following key words: aconitine, arrhythmia, infusion, and rats. This search was conducted on December 22, 2015 and covers the years 1975 through the present (31 publications found, all of which confirm these statements).

## **SECTION C - Animal requirements**

***Describe the following animal requirements :***

**1. Indicate the number of animals required over the study period for this protocol. Please enter numbers only.**

**a. Animals to be purchased from a Vendor for this study:** 240

**b. Animals to be transferred from another LAPR:  
LAPR Number that is the source of this**

**transfer:**

**c. Animals to be transferred from another source:**

**d. Offspring produced onsite (used for data collection and/or weaned):**

**e. TOTAL NUMBER of animals for duration of the LAPR** 240

**2. Species (limited to one per LAPR):** Rat(s)

**3. Strain:** WKY, Sprague Dawley, p75 -/  
**Describe special requirements for animals with altered physiological responses (e.g., genetically altered, aged)**

**4. Sources of animals:**

Charles River Laboratories for WKY rats and Horizon for p75 -/- and Sprague Dawley rats (I will work with both the AV and ARPO well before any submission of requests for p75-/- rat procurement to ensure that all biosecurity concerns are met)

**5. Provide room numbers where various procedures will be performed on animals:**

Air Pollution exposures in Building A

- CAPs Exposures: Exemption 6
- Wood Smoke Exposures: Exemption 6
- Housing and telemetric monitoring for animals exposed in Building A: Exemption 6
- Aconitine Challenge for animals exposed in Building A: Exemption 6
- Euthanasia for animals exposed in Building A: Exemption 6

Smog and Diesel Exhaust Exposures: Approved satellite facility Exemption 6

- Housing, telemetric monitoring, oral gavage of statin: Exemption 6
- Oral gavage of fat emulsion, ultrasound, and euthanasia for animals exposed in High Bay: Exemption 6

Note: Animals exposed in the High Bay will not be brought back into Building A.

**6. Will any animals be housed in areas other than the animal facility longer than 12 hours? If so, state location. Such areas require prior IACUC approval as a satellite facility before LAPR can be**

reviewed.

Yes. Rats will be transferred to the approved satellite housing/exposure facility **Exemption 6** for smog or diesel exhaust exposures. **Room Numbers** **Exemption 6**

**7. Describe any transportation and containment methods involved in moving animals between EPA buildings, or between EPA and other institutions (excluding any commercial shipments)**

Standard polycarbonate cages with pine shavings bedding and a water bottle should be used for transfer between EPA buildings

**8. Describe any unusual housing or husbandry requirements, or acclimation requirements. Justify any treatment beginning less than 3 days after arrival.**

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**9. Describe special assistance requested of the animal contract staff, including procedures and dosing. NOTE, this request must be submitted separately to the Animal Resources Program Office (ARPO)**

The animal care staff will be requested to perform oral gavages and identity chip implants.

**10. Housing and Enrichment.**

*The IACUC encourages the use of environmental enrichment whenever possible (see IACUC website for details). Provide details on how the animals will be housed, including type of cage (e.g., solid bottom or wire screen), bedding material, number of animals per cage, and environmental enrichment. Note that housing rodents individually without environmental enrichment requires justification.*

All unimplanted rats will be housed 2 per cage. Rats that are implanted with telemeters must be singly housed because of the strong likelihood of signal crosstalk/interference among signals from two or more telemeters. Singly-housed rats with implanted telemeters will be provided with EnviroDri for enrichment.

**SECTION D - Agents Administered to Animals**

**1. Identify all hazardous and non-hazardous agents to be administered to living animals. For agents requiring a Health and Safety Research Protocol (HSRP), provide the title of the approved HSRP for each such agent. If no protocol is required for an agent deemed potentially hazardous (e.g. nanoparticles, recombinant DNA), describe the safety precautions to be used. Provide maximum dosing levels and route-appropriate LD50s (where available) for each agent used for dosing.**

**Air Pollutants**

1. Concentrated Ambient Particulate matter (PM): ambient PM. The maximum exposure concentration for Pm = 500 micrograms/m<sup>3</sup> inhalation studies; no LC50 known or available.

2. Nitrogen dioxide (NO<sub>2</sub>): The maximum exposure concentration for NO<sub>2</sub> will be 0.5 ppm. The rat inhalation LC50 for NO<sub>2</sub> is 115 ppm for 1 hour and 88 ppm for 4 hours.

Rodent diet with 10, or 60 kcal% Fat (from Research Diets) suspended in water: contains full complement of vitamins, minerals, carbohydrates, protein and fat. For the 60 kcal% emulsion, 60 percent of the calories are derived from fat (see attachment). There is no known LD50. The maximum dose will be 10 ml/kg of 1 kcal/ml.

Aconitine (C<sub>34</sub>H<sub>47</sub>NO<sub>11</sub>): LD50 in rats = 5.97 mg/kg (oral); 80 ug/kg (intravenous). Maximum dose = 60 ug/kg (intravenous). HSRP: Title: "ARRHYTHMIA SUSCEPTIBILITY FOLLOWING EXPOSURE TO AIR TOXICS IN WISTAR-KYOTO, SPRAGUE DAWLEY AND SPONTANEOUSLY HYPERTENSIVE RATS"

Saline: no known LD50; maximum volume = 2 ml

Pitavastatin: Rat Oral LD50 = 3200 mg/kg: Maximum dose: 5 mg/kg (via oral gavage). A 5 mg/ml solution of pitavastatin in carboxymethylcellulose will be prepared. Thus, a 300 gram rat will receive 0.3 ml of the pitavastatin solution.

Carboxymethylcellulose (vehicle for pitavastatin): Rat Oral LD50 = 27000 mg/kg. Maximum concentration will be 0.5%. Maximum volume will be 500 microliters. Maximum dose = 2.5 mg.

#### Analgesics/Anesthetics

1. Ketamine/xylazine: Maximum dose of ketamine/xylazine to be administered 1-2 ml/kg of an 80 mg/ml ketamine/12 mg/ml xylazine solution. Ketamine LD50 rat, oral = 447 mg/kg. LD50 rat, intraperitoneal = 224 mg/kg. Xylazine LD50 rat, oral = 130 mg/kg.
2. Sodium pentobarbital/phenytoin: Maximum dose of Na pentobarbital to be administered = 200 mg/kg; Maximum dose of Phenytoin to be administered = 25 mg/kg. Pentobarbital LD50 rat, oral = 118 mg/kg. Phenytoin LD50 rat, oral = 1530 mg/kg
3. Buprenorphine: Maximum concentration = 0.05 mg/kg. LD50 rat, oral = 243 mg/kg
4. Urethane: Maximum dose to be administered is 1.5 mg/kg. LD50 rat, oral = 1809 mg/kg
5. Isoflurane: Maximum concentration is 3%. LC50 rat, inhalation = 15,300 ppm. Isoflurane is considered a "potentially hazardous substance" but does not require an HSRP. Isoflurane will be used in the chemical safety hood in A-579 or A-587, and standard PPE (safety glasses, gloves, lab coat) will be worn by all personnel at all times while being used.

#### **2. Describe compounds to be administered to animals.**

**a. Are all substances pharmaceutical grade? If not, provide a scientific justification for the use of non pharmaceutical grade compounds.**

All of the agents in section D1 above will be pharmaceutical grade except the air pollutants, aconitine and urethane, which are unavailable as pharmaceutical grade.

**b. Describe any plans to administer human or animal tissues, blood or body fluids to the animals in the LAPR. Provide information to assure that such material is pathogen free. Indicate what safety precautions are necessary for handling the material.**

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**c. Provide a statement regarding any safety precautions necessary for handling any of these materials.**

Normal personal protective equipment (PPE) precautions will be observed throughout (gloves mask, labcoat, safety glasses). Inhalation exposures for wood smoke will be conducted under negative pressure to ensure safety of personnel. For CAPS-Acrolein studies, the nose-only tubes form a tight seal with the ports of the nose-only tower preventing release of escape acrolein gas. In addition, the nose-only towers will be placed in a secondary chamber with an exhaust to capture released of any gas.

**NOTE: Any unresolved health/safety questions which arise during IACUC review of this LAPR will require consultation with the Safety, Health, and Environmental Management Office.**

### **SECTION E - Personnel Training and Experience**

**1. Identify all project personnel conducting animal experimentation. Specify the techniques for which they have responsibility, and their relevant training and experience. Additional personnel may be added to the table below as a group (by Division) for Category C procedures. By so doing you are giving assurance that these personnel have received all required training and are qualified to perform the Category C techniques requested.**

**Use this area to type in additional personnel information not available in the table drop-down lists:**

**Hint:** The names in the first 2 lines of the table below are filled automatically from the Principal Investigator & Alternate Contact fields. A new line will be made available when a name is selected & upon leaving the name field (i.e. tabbing or clicking in another field).

NAME	ROLE	SPECIFIC RESPONSIBILITY	RELEVANT TRAINING
Exemption 6	Principal Investigator	study coordination; animal handling/care; surgical procedures; physiological monitoring via radiotelemetry	17 years of experience in use of laboratory animals; > 9 years surgical procedures and physiological monitoring; completed NHEERL animal use training
Exemption 6	Associate Principal Investigator	study coordination, pulmonary function monitoring; surgical procedures; physiological monitoring via radiotelemetry, arrhythmia testing;	13 years of experience in use of laboratory animals; 10 years of experience in surgical procedures and physiological monitoring; completed NHEERL training
Exemption 6	Associate Principal Investigator	study coordination, euthanasia, post-mortem systemic assessments	>20 years of experience in use of laboratory animals; completed all NHEERL training
Exemption 6	Technical Staff	animal handling/care; surgical procedures; physiological monitoring via radiotelemetry	>20 years of experience in use of laboratory animals and pulmonary function monitoring; 6 years, surgical procedures; completed NHEERL training
Exemption 6	Post-Doc	animal handling/care; ; physiological monitoring; euthanasia	> 2 year echo exp, completed all NHEERL training, >6 years experience in use of lab animals
Exemption 6	Student	animal handling/care; ; physiological monitoring; euthanasia	completed all NHEERL training, 2 years experience in use of lab animals
Exemption 6	Technical Staff	animal handling/care; surgical procedures; physiological monitoring via radiotelemetry	>20 years of experience in use of laboratory animals and pulmonary function monitoring; 6 years, surgical procedures; completed NHEERL training
Exemption 6	Technical Staff	animal handling, whole body inhalation procedures	>20 years of experience in use of laboratory animals and inhalation exposures; has completed NHEERL training
Exemption 6	Technical Staff	animal handling, whole body inhalation procedures	>20 years of experience in use of laboratory animals and inhalation exposures; has completed NHEERL training
Exemption 6	Technical Staff	animal handling, whole body inhalation procedures	>20 years of experience in use of laboratory animals and inhalation exposures; has completed NHEERL training
RTP-NHEERL	Tech Support	Category C Procedures	All NHEERL required training is complete.

## SECTION F - Animal Breeding Colonies

***This section pertains to the breeding of animals for maintenance of ongoing animal colonies. Do not include breeding that is part of experimentation and accountable under Section C.***

***Describe:***

- 1. Estimated number of breeding pairs and liveborn per year***
- 2. Breeding protocols and recordkeeping***
- 3. Methods for monitoring genetic stability***
- 4. Disposition of all offspring and retired breeders that are not used in accordance with the procedures described in this LAPR***

## **SECTION G - Euthanasia**

### ***1. When will the animals be euthanized relative to experimental procedures?***

All animals will be euthanized following completion of experimental procedures (approximately 1 day after final air pollutant exposure).

### ***2. Describe the euthanasia techniques:***

***Method(s):*** Anesthesia plus exsanguination, Anesthesia plus vital organ transection  
***Agent(s):*** Sodium pentobarbital: 200 mg/kg; Phenytoin: 25 mg/kg  
***Dose (mg/kg):*** Overdose of pentobarbital (200 mg/kg) followed by transection of abdominal aorta and vital organs  
***Volume:*** Approximately 0.25 ml/rat for a 250 g rat (200 mg/ml solution)  
***Route:*** Intraperitoneal

***Source(s) of information used to select the above agents/methods:***

- 2013 AVMA Guidelines on Euthanasia.

***3. Provide justification and references for any euthanasia agent or method that is not consistent with recommendations of the American Veterinary Medical Association (AVMA) Guidelines for Euthanasia (e.g., cervical dislocation or decapitation without anesthesia; cervical dislocation in rodents weighing more than 200 grams).***

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### ***4. Describe how death is to be confirmed.***

Vital organ section

## **SECTION H - Disposition of Used and Unused Animals**

***Describe the disposition of any animals remaining after project completion.***

Euthanized as above

Euthanized by Animal Care Contractor

Transferred to another study

***The IACUC encourages investigators to reduce the overall number of animals used at NHEERL. Would you consider transferring any unused animals from this LAPR to another approved LAPR?***

☒ Yes ☐ No

## **SECTION I - Assurances**

***1. Animals will not be used in any manner beyond that described in this application without first obtaining formal approval of the IACUC.***

2. All individuals involved in this project have access to this application, are aware of all EPA policies on animal care and use, and are appropriately trained and qualified to perform the techniques described.
3. Thorough consideration of the three "R"'s (Replacement, Reduction, Refinement) has been given, as applicable, to a. the use of animals, and b. procedures causing pain or distress (with or without analgesia/anesthesia), including death as an endpoint. The minimum number of animals required to obtain valid experimental results will be used.
4. The Attending Veterinarian has been consulted in regard to any planned experimentation involving pain or distress to animals.
5. The IACUC and Attending Veterinarian will be promptly notified of any unexpected study results that impact the animals' well-being, including morbidity, mortality and any occurrences of clinical symptoms which may cause pain or indicate distress.
6. All procedures involving hazardous agents will be conducted in accordance with practices approved by the Safety, Health, and Environmental Management Office.
7. I certify that I am familiar with and will comply with all pertinent institutional, state and federal rules and policies.
8. The IACUC has oversight responsibilities for animal care and use, and may request consultation or feedback regarding the conduct of in vivo procedures, progress and accomplishments, and any problems encountered.

EPA Principal Investigator	Certification Signature Date
Exemption 6 Exemption 6	11/17/2015





Submitted: 11/17/2015

#### Certification:

Certification by EPA Supervisor (Branch Chief or Division Director) that the project described herein has been reviewed and approved on the basis of scientific merit:

Branch Chief/Division Director	Approval Date	Phone Number	Division	Mail Drop
Exemption 6	11/19/2015	Exemption 6 Lotus Notes Address	EPHD Branch	MD Submitted to Branch Chief for Approval
by Exemption 6 Exemption 6 Exemption 6	/RTP/USEP A/US	Exemption 6 /RTP/USEP A/US	CIB	11/17/2015 03:34 PM

#### ATTACHMENTS

-  18-11-002 PI response.pdf Adebisi and Abatan-2013\_Acute toxicity of Enantia chlorantha ethanol extract in Rats.pdf
-  Hadley et al-2010\_Safety evaluation of Algal-Docosahexaenoic Acid Ester in Rats.pdf
-  McConnell et al, 2008 - Rat and mouse GI pH, fluid, and lymphoid tissue and implications for in-vivo experiments .pdf
-  18-11-02 Prescreen PI response.pdf Approved Exemption 6  
Exemption 6 IRP\_\_IRP-NHEERL-RTP-EPHD-CIB Exemption 6 15-01-001.pdf



Charles River Blood Pressure Telemetry - Implant Procedure and IACUC Approval.pdf



Research Diets\_60kcal%\_Fat\_D12492L.pdf

## Actions

*First Update notification sent: 12/09/2016*

*Second Update notification sent:*

*First 2nd Annual notification sent:*

*12/04/2017*

*Second 2nd Annual notification sent:*

*1st Expiration notification sent:*

*2nd Expiration notification sent:*

## History Log: